

Application No.: 10/810733
Docket No.: CL2385USNA

Page 2

Amendments to Claims

Claim 1 (Withdrawn) An isolated nucleic acid molecule encoding a carotenoid biosynthetic pathway enzyme, selected from the group consisting of:

- (a) an isolated nucleic acid molecule encoding the amino acid sequence selected from the group consisting of SEQ ID NOs:2, 4, 6, 8, 10, 12 and 14;
- (b) an isolated nucleic acid molecule that hybridizes with (a) under the following hybridization conditions: 0.1X SSC, 0.1% SDS, 65°C and washed with 2X SSC, 0.1% SDS followed by 0.1X SSC, 0.1% SDS; and
- (c) an isolated nucleic acid molecule that is complementary to (a) or (b).

Claim 2 (Withdrawn) The isolated nucleic acid molecule of Claim 1 selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11 and 13.

Claim 3 (Withdrawn) An isolated nucleic acid fragment of Claim 1 isolated from *Pantoea stewartii* strain DC413.

Claim 4 (Withdrawn) A polypeptide encoded by the isolated nucleic acid molecule of Claim 4.

Claim 5 (Withdrawn) The polypeptide of Claim 7 selected from the group consisting of SEQ ID NOs:2, 4, 6, 8, 10, 12 and 14.

Claim 6 (Canceled)

Claim 7 (Withdrawn) An isolated nucleic acid molecule comprising a first nucleotide sequence encoding a geranylgeranyl pyrophosphate synthetase enzyme of at least 302 amino acids that has at least 70% identity based on the Smith-Waterman method of alignment when compared to a polypeptide having the sequence as set forth in SEQ ID NO:2; or a second nucleotide sequence comprising the complement of the first nucleotide sequence.

Claim 8 (Withdrawn) An isolated nucleic acid molecule comprising a first nucleotide sequence encoding an isopentenyl pyrophosphate isomerase enzyme of at least 344 amino acids that has at least 70% identity based on the Smith-Waterman method of alignment when compared to a polypeptide having the sequence as set forth in SEQ ID NO:4; or a second nucleotide sequence comprising the complement of the first nucleotide sequence.

Claim 9 (Withdrawn) An isolated nucleic acid molecule comprising a first nucleotide sequence encoding an crtX enzyme of at least 429 amino acids that has at least 70% identity based on the Smith-Waterman method of alignment when compared to a polypeptide having the sequence as set forth in SEQ ID NO:6; or a second nucleotide sequence comprising the complement of the first nucleotide sequence.

Claim 10 (Withdrawn) An isolated nucleic acid molecule comprising a first nucleotide sequence encoding a lycopene cyclase enzyme of at least 387 amino acids that has

Application No.: 10/810733
Docket No.: CL2385USNA

Page 3

at least 70% identity based on the Smith-Waterman method of alignment when compared to a polypeptide having the sequence as set forth in SEQ ID NO:8;

or a second nucleotide sequence comprising the complement of the first nucleotide sequence.

Claim 11 (Withdrawn) An isolated nucleic acid molecule comprising a first nucleotide sequence encoding a phytoene desaturase enzyme of at least 493 amino acids that has at least 81% identity based on the Smith-Waterman method of alignment when compared to a polypeptide having the sequence as set forth in SEQ ID NO:10;

or a second nucleotide sequence comprising the complement of the first nucleotide sequence.

Claim 12 (Withdrawn) An isolated nucleic acid molecule comprising a first nucleotide sequence encoding a phytoene synthase enzyme of at least 309 amino acids that has at least 70% identity based on the Smith-Waterman method of alignment when compared to a polypeptide having the sequence as set forth in SEQ ID NO:12;

or a second nucleotide sequence comprising the complement of the first nucleotide sequence.

Claim 13 (Withdrawn) An isolated nucleic acid molecule comprising a first nucleotide sequence encoding a β -carotene hydroxylase enzyme of at least 177 amino acids that has at least 82% identity based on the Smith-Waterman method of alignment when compared to a polypeptide having the sequence as set forth in SEQ ID NO:14;

or a second nucleotide sequence comprising the complement of the first nucleotide sequence.

Claim 14 (Withdrawn) A chimeric gene comprising the isolated nucleic acid molecule of any one of Claims 1 or 7-13 operably linked to suitable regulatory sequences.

Claim 15 (Original) A vector comprising the isolated nucleic acid molecule of Claim 6.

Claim 16 (Withdrawn) A transformed host cell comprising the chimeric gene of Claim 14.

Claim 17 (Original) A transformed host comprising the isolated nucleic acid molecule of claim 6.

Claim 18 (Currently Amended) The transformed host cell of Claim 16 or 17 wherein the host cell is selected from the group consisting of bacteria, yeast, filamentous fungi, and algae, ~~and green plants~~.

Claim 19 (Currently Amended) The transformed host cell of Claim 16 or 17 wherein the host cell is selected from the group consisting of *Aspergillus*, *Trichoderma*, *Saccharomyces*, *Pichia*, *Candida*, *Hansenula*, *Yarrowia*, *Rhodospiridium*, *Lipomyces*, *Salmonella*, *Bacillus*, *Acinetobacter*, *Zymomonas*, *Agrobacterium*, *Flavobacterium*, *Rhodobacter*, *Rhodococcus*, *Streptomyces*, *Brevibacterium*, *Corynebacteria*, *Mycobacterium*,

Application No.: 10/810733
Docket No.: CL2385USNA

Page 4

Escherichia, Pantoea, Pseudomonas, Methylomonas, Methylobacter, Methylococcus, Methylosinus, Methylobacterium, Methylocystis, Alcaligenes, Synechocystis, Synechococcus, Anabaena, Thiobacillus, Methanobacterium, Klebsiella, Methylophilus, Methylobacillus, Methylobacterium, Hyphomicrobium, Xanthobacter, Paracoccus, Nocardia, Arthrobacter, Rhodopseudomonas, Torulopsis, Phaffia, and Rhodotorula.

Claim 20 (Withdrawn) A method for the production of carotenoid compounds comprising:

- (a) providing a transformed host cell comprising:
 - (i) suitable levels of farnesyl pyrophosphate; and
 - (ii) a set of nucleic acid molecules encoding the enzymes selected from the group consisting of SEQ ID NOs:2, 4, 6, 8, 10, 12 and 14 under the control of suitable regulatory sequences;
- (b) contacting the host cell of step (a) under suitable growth conditions with an effective amount of a fermentable carbon substrate whereby a carotenoid compound is produced.

Claim 21 (Withdrawn) A method for the production of carotenoid compounds comprising:

- (a) providing a transformed host cell comprising:
 - (i) suitable levels of farnesyl pyrophosphate; and
 - (ii) a the isolated nucleic acid molecule of claim 6 under the control of suitable regulatory sequences;
- (b) contacting the host cell of step (a) under suitable growth conditions with an effective amount of a fermentable carbon substrate whereby a carotenoid compound is produced.

Claim 22 (Withdrawn) A method according to Claim 20 or 21 wherein the transformed host cell is selected from the group consisting of C1 metabolizing hosts, bacteria, yeast, filamentous fungi, algae, and green plants.

Claim 23 (Withdrawn) A method according to Claim 22 wherein the C1 metabolizing host is a methanotroph and the fermentable carbon substrate is selected from the group consisting of methane, methanol, formaldehyde, formic acid, methylated amines, methylated thiols, and carbon dioxide.

Claim 24 (Withdrawn) A method according to Claim 23 wherein the C1 metabolizing host:

- (a) grows on a C1 carbon substrate selected from the group consisting of methane and methanol; and
- (b) comprises a functional Embden-Meyerhof carbon pathway, said pathway comprising a gene encoding a pyrophosphate-dependent phosphofructokinase enzyme.

Application No.: 10/810733
Docket No.: CL2385USNA

Page 5

Claim 25 (Withdrawn) A method according to Claim 24 wherein the C1 metabolizing host cell is a high growth methanotrophic bacterial strain, known as *Methylomonas* 16a and having the ATCC designation PTA 2402.

Claim 26 (Withdrawn) A method according to Claim 20 or 21 wherein the transformed host cell is selected from the group consisting of *Aspergillus*, *Trichoderma*, *Saccharomyces*, *Pichia*, *Candida*, *Hansenula*, *Yarrowia*, *Rhodospiridium*, *Lipomyces*, *Salmonella*, *Bacillus*, *Acinetobacter*, *Zymomonas*, *Agrobacterium*, *Flavobacterium*, *Rhodobacter*, *Rhodococcus*, *Streptomyces*, *Brevibacterium*, *Corynebacteria*, *Mycobacterium*, *Escherichia*, *Pantoea*, *Pseudomonas*, *Methylomonas*, *Methylobacter*, *Methylococcus*, *Methylosinus*, *Methylomicrobium*, *Methylocystis*, *Alcaligenes*, *Synechocystis*, *Synechococcus*, *Anabaena*, *Thiobacillus*, *Methanobacterium*, *Klebsiella*, *Methylophilus*, *Methylobacillus*, *Methylobacterium*, *Hyphomicrobium*, *Xanthobacter*, *Paracoccus*, *Nocardia*, *Arthrobacter*, *Rhodopseudomonas*, *Torulopsis*, *Phaffia*, and *Rhodotorula*.

Claim 27 (Withdrawn) A method according to Claim 20 or 21, wherein the carotenoid compound produced is selected from the group consisting of antheraxanthin, adonirubin, adonixanthin, astaxanthin, canthaxanthin, capsorubrin, β -cryptoxanthin, α -carotene, β -carotene, epsilon-carotene, echinenone, 3-hydroxyechinenone, 3'-hydroxyechinenone, γ -carotene, 4-keto- γ -carotene, ζ -carotene, α -cryptoxanthin, deoxyflexixanthin, diatoxanthin, 7,8-didehydroastaxanthin, fucoxanthin, fucoxanthinol, isorenieratene, lactucaxanthin, lutein, lycopene, myxobactone, neoxanthin, neurosporene, hydroxyneurosporene, peridinin, phytoene, rhodopin, rhodopin glucoside, 4-keto-rubixanthin, siphonaxanthin, spheroidene, spheroidenone, spirilloxanthin, 4-keto-torulene, 3-hydroxy-4-keto-torulene, uriolide, uriolide acetate, violaxanthin, zeaxanthin- β -diglucoside, and zeaxanthin.

Claim 28 (Withdrawn) A method of regulating carotenoid biosynthesis in an organism comprising over-expressing at least one carotenoid biosynthetic pathway gene selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13 and 20 in an organism such that the carotenoid biosynthesis is altered in the organism.

Claim 29 (Withdrawn) A method according to Claim 28 wherein said carotenoid gene is over-expressed on a multicopy plasmid.

Claim 30 (Withdrawn) A method according to Claim 28 wherein said carotenoid gene is operably linked to an inducible or regulated promoter.

Claim 31 (Withdrawn) A method according to Claim 28 wherein said carotenoid gene is expressed in antisense orientation.

Claim 32 (Withdrawn) A method according to Claim 28 wherein said carotenoid gene is disrupted by insertion of foreign DNA into the coding region.

Claim 33 (Withdrawn) A strain DC413 comprising the 16S rDNA sequence as set forth in SEQ ID NO:18.

Application No.: 10/810733
Docket No.: CL2385USNA

Page 6

Claim 34 (Withdrawn) An isolated nucleic acid molecule encoding all of the amino acid sequences as set forth in SEQ ID NO:2, 4, 6, 8, 10, 12, and 14.

Claim 35 (Withdrawn) The isolated nucleic acid molecule of claim 40 having the nucleic acid sequence as set forth in SEQ ID NO:20.

Claim 36 (New) An isolated nucleic acid molecule having the nucleic acid sequence as set forth in SEQ ID NO:20.

Claim 37 (New) An isolated nucleic acid molecule having at least 95% sequence identity to SEQ ID NO:20 and encoding all of the following enzymes:

- a) geranylgeranyl pyrophosphate synthetase;
- b) zeaxanthin glucosyl transferase;
- c) lycopene cyclase;
- d) phytoene desaturase;
- e) phytoene synthase;
- f) β -carotene hydroxylase; and
- g) isopentenyl diphosphate isomerase.